Synopsis

Enhancing *In Vitro* Propagation Efficiency and Exploring Wound Healing Therapeutic Potential of *Rauvolfia tetraphylla* L.: A Multifaceted Approach

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Enhancing *In vitro* Propagation Efficiency and Exploring Wound Healing Therapeutic Potential of *Rauvolfia tetraphylla* L.: A Multifaceted Approach

Rauvolfia tetraphylla L. adapts to diverse environmental conditions across its natural range. *R. tetraphylla* a plant belonging to the Apocynaceae family, is indigenous to India and Southeast Asia. Renowned for its medicinal attributes, this botanical gem has garnered attention for its role in traditional healing practices. The plant's therapeutic efficacy stems from its rich reservoir of bioactive compounds, particularly indole alkaloids such as reserpine. These compounds exhibit a spectrum of pharmacological activities, including antihypertensive, sedative, antipsychotic, and anti-cancer properties. Beyond its medicinal utility, *R. tetraphylla* has diverse uses. Its sturdy wood finds application in crafting tool handles. Additionally, the plant's ornamental appeal, characterized by evergreen foliage and fragrant flowers, enhances its cultural significance.

However, due to overexploitation *Rauvolfia tetraphylla* has fallen in the category of endangered plant and present investigations have focused on two aspects: to develop and optimize *in vitro* method of propagation, conservation and understand alkaloid biosynthesis pathways. Traditional knowledge about wound healing properties of this plant has been explored employing biochemical and pharmaceutical parameters**.** Consequently, this research has the potential to address the therapeutic gap crucial for effective wound healing.

Thus, our research endeavour comprises two distinct objectives, yet interconnected research statements aimed at addressing critical gaps in *R. tetraphylla* plant research. Its therapeutic potential extends to combating hypertension, ulcers, and mental disorders. It roots extracts serves as an antidote for snake bites and insect stings, underlining its importance in folk medicine. *Rauvolfia tetraphylla* emerges as a botanical powerhouse with profound medicinal and cultural significance. Rich phytochemical composition of *R. tetraphylla*, coupled with its adaptability to varied habitats, underscores its role as a valuable asset in traditional healing practices and biodiversity conservation efforts.

The protocol for micropropagation using embryogenesis has been developed for *Rauvolfia tetraphylla*, potentially revolutionizing botanical conservation and mass multiplications efforts. Detailed investigations into the molecular mechanisms behind alkaloid biosynthesis, helped to

unravel fundamental processes that could have far-reaching implications in both pharmaceutical and agricultural industries.

The wound-healing potential of *R. tetraphylla* has been empirically investigated and substantiated bridging the gap between traditional knowledge and contemporary scientific inquiry. These studies unveiled novel therapeutic avenues for wound management, contributing significantly to the field of herbal medicine and pharmacology.

Present studies are multifaceted: blending traditional wisdom with rigorous scientific inquiry, ultimately aiming to unlock the latent potential of plant-based remedies for human welfare and ecological sustainability.

Objectives

- To optimization of seed germination techniques, callus formation, micropropagation and histological aspects of somatic embryogenesis derived from the callus.
- Phytochemical characterization of plant extracts to determine phytochemicals, followed by identification utilizing GCMS and LCMS data and characterized the compound by column chromatography techniques.
- The extracts from *Rauvolfia tetraphylla* assessments for various biological activities to evaluate their wound healing properties.
- To isolate and characterize the Tryptophan Decarboxylase (TDC) gene from *Rauvolfia tetraphylla* (*RtTDC*) and analyse its sequence, structure, catalytic regions, evolutionary relationships, physicochemical properties, and substrate recognition was followed by experimental validation of its catalytic activity through a TDC assay.
- To exhibit docking analysis of the bioactive compounds of *Rauvolfia tetraphylla* against a wound healing protein (6Y8M, 6B8Y and 1GEN).

The use of medicinal plants has been deeply rooted in human history, dating back to ancient civilizations such as China, Greece, Egypt, and India. Throughout the ages, medicinal plants have been integral to traditional herbal medicines, providing vital therapeutic properties for treating various ailments. Over time, the understanding of the bioactive compounds within medicinal plants has evolved, leading to the isolation and synthesis of these compounds for modern therapeutic use.

Today, medicinal plants continue to play a crucial role in both traditional and modern medicine. With over 80,000 plant species recognized for their medicinal value, these botanical resources offer a diverse array of bioactive compounds with health-promoting properties. India boasts a rich biodiversity of medicinal plants, contributing significantly to traditional systems of medicine such as Ayurveda and Unani.

Advent of plant tissue culture has revolutionized the micropropagation of endangered plants, plant breeding, genetic engineering, overcome the seed germination, and the production of secondary metabolites. It enables the efficient propagation of plants, contributing to agricultural and pharmaceutical industries.

Phytochemistry is the study of chemical compounds in plants and serves as the foundation for drug development. The exploration of antimicrobial, antioxidant, cytotoxic, and anti-inflammatory activities of medicinal plant compounds present promising avenues for therapeutic interventions. Moreover, molecular biology techniques have enhanced our understanding of plant genetics and facilitated the isolation and manipulation of DNA for various applications. Molecular studies, including molecular docking and computational analysis, provide valuable insights into the interactions between bioactive compounds and biological targets, aiding in drug discovery and development.

In this present study, seed dormancy was overcome by this efficient technique. Higher percentages (98.44%) of sterilized plantlets of *R. tetraphylla* were obtained using sodium hypochlorite and mercuric chloride solutions. Maximum germination percentage was achieved at 20 minutes of $NaHCl_3$ followed by 3 minutes of $HgCl_2$ treatment.

A combination of surface sterilization, cold treatment, and partial removal of the seed coat significantly enhanced the germination rates under *in vitro* conditions. Cold-treated, pre-cut seeds exhibited 83.33% germination on agar-solidified MS medium containing 3% sucrose, over uncut seeds which remained dormant conditions. It emphasizes the importance of addressing seed coat impermeability to enhance germination rates in *R. tetraphylla* seeds under controlled conditions. By overcoming the barrier imposed by the hard seed coat, the technique facilitates seed germination and seedling development, providing a valuable method for propagating *R. tetraphylla* for further research.

Influence of different plant growth regulators i.e., IAA, IBA, 2,4-D, and BAP and their combinations on callus formation of various explants are leaves, node, internode, and root explants of *R. tetraphylla* elaborate experimentation was done.

Gradual increases in the callus formation were observed with increased concentrations of individual growth regulators. Combinations of hormones further enhanced callus induction compared to individual growth regulators. The maximum callus formation in the leaf (88.62%), node (83.45%), internode (79.66%) and root (84.11%) explants of *R. tetraphylla* was achieved on MS medium with a combination of 2,4-D + BAP (2.5 mg/L each). These results provide valuable insights for optimizing tissue culture protocols and enhancing the efficiency of callus formation and micropropagation in plant tissue culture systems. It demonstrates the significant influence of specific hormone combinations and concentrations on callus formation of *R. tetraphylla*. By understanding the intricate relationships between hormones and callus formation, we can tailor protocols to maximize callus induction rates, thereby advancing plant tissue culture techniques and biotechnological applications in plant science.

Analysed the Direct organogenesis of root induction from leaves and nodal explants of *R. tetraphylla* on MS medium with different concentration of PGRs (IAA, 2,4-D, IBA, and BAP). Increasing concentrations of IAA led to higher percentages of direct root formation, with a similar trend observed for hormonal combinations of 2,4-D+BAP, IAA+BAP, and IBA+BAP. The maximum percentages of direct root formation from leaf (87.50%) and node (83.16%) were achieved on MS medium with the combinations of IAA (1.5 mg/L) + BAP (0.5 mg/L). It emphasizes the significant influence of different plant growth hormones and their concentrations on direct root formation in leaf and nodal explants of *R. tetraphylla*.

The influence of different hormone concentrations on direct shooting and multiple shoot formation from nodal explants of *R. tetraphylla* were investigated on MS medium with BAP, 2,4-D+BAP, IAA+BAP, and KIN+BAP. Results indicated that increasing concentrations of BAP had a significant impact on both direct shooting and multiple shoot formation. Similarly, combinations of 2,4-D+BAP, IAA+BAP, and KIN+BAP also demonstrated positive effects on both shooting parameters. The maximum percentage of direct shoot (83.44%) and multiple shoot (88.44%) was achieved on MS medium with IAA + BAP (1.5 mg/L each). It indicates the importance of hormone

concentration optimization in promoting direct and multiple shoot formation of *R. tetraphylla* nodal explants.

The impacts of various PGRs on somatic embryogenesis in leaf and root explants were evaluated and the preliminary experiments identified 2,4-D and BAP as the most effective auxin and cytokinin at an optimal concentration of 2.5 mg/L. Further experiments focused on leaf and root explants cultured on MS medium with different concentrations of 2,4-D + BAP. After a 4-week culture period, the maximum embryogenesis was observed with a combination of BAP + 2,4-D (1.5 mg/L), indicating a synergistic effect conducive for somatic embryo development. This established the potential for achieving plant regeneration through direct somatic embryogenesis, offering insights into optimizing tissue culture protocols for efficient somatic embryo production.

The histological analysis of *R. tetraphylla* during somatic embryogenesis and callus formation provided insights into the cellular processes underlying plant regeneration. During somatic embryogenesis, embryogenic cells at the outer layer exhibited characteristics conducive to embryo development, while non-embryogenic cells in the inner regions displayed distinct features. Maturation stages showed the differentiation of somatic embryos, marked by the formation of protoderms and the initiation of pro-embryogenesis. The transition from globular to torpedo-shaped embryos involved the development of vascular tissue, root and shoot primordia. Additionally, increased endogenous sugars, particularly starch, were observed in leaf and root explants contributing to cellular protection during dehydration and freezing.

Histological analysis elucidated the cellular dynamics during somatic embryogenesis in *R. tetraphylla*, shedding light on the progression of embryogenic and non-embryogenic cell populations. The identification of distinct cell types and developmental stages provides a foundation for understanding the mechanisms underlying *in vitro* plant regeneration.

The phytochemical analyses of *R. tetraphylla* leaf and fruit extracts using different solvents revealed the presence of a diverse array of secondary metabolites. These included alkaloids, carbohydrates, glycosides, oils and fats, phenolic compounds, tannins, quinones, cardiac glycosides, terpenoids, coumarins, phlobatannins, steroids, phytosteroids, anthraquinones, flavonoids, and proteins across all the extracts. The detection of various secondary metabolites in *R. tetraphylla* leaf and fruit extracts suggested their potential pharmacological and therapeutic

significance. These compounds may contribute to the determination of medicinal properties associated with *R. tetraphylla*, which corroborated its traditional uses in herbal medicine.

The tannin, alkaloid, flavonoid, and total phenolic content of *R. tetraphylla* leaf and fruit extracts were determined using ethyl acetate and methanol crude extracts for different chemical assays. Gallic acid (tannins and phenols), atropine (alkaloids), and rutin (flavonoids) were used as standard. The maximum tannin (33.92 µg/mg), alkaloids (2.10µg/mg), and flavonoids (1.92 µg/mg) content was observed in the methanolic extract of leaf, and the maximum total phenolic content (22.88 µg/mg) was identified in the methanolic extract of fruit. This provided valuable insights into the phytochemical composition of *R. tetraphylla*, establishing, and highlighting its potential pharmacological and therapeutic properties. The presence of bioactive compounds such as tannins, alkaloids, flavonoids, and phenols suggest that *R. tetraphylla* may possess antioxidant, antimicrobial, and other health-promoting properties. Further research is warranted to explore the biological activities and medicinal applications of these phytochemicals, as well as their potential synergistic effects. Additionally, the variation in phytochemical content among different plant parts and extraction solvents underscores the importance of standardized extraction protocols for obtaining consistent and reliable results in phytochemical analysis.

The GC-MS analysis of the methanolic extract of *R. tetraphylla* exhibited the presence of twelve compounds, each with specific percentages of abundance. These compounds include 1,9-Nonanediol, 3-Furanol, tetrahydro-2,2,4,4-tetramethyl-, 1D-1-O-Methyl-myo-inositol, (Z)-2-methylbutanal oxime, Arabino-heptitol, 2,3:5,6-dianhydro-1,7-dideoxy-2,6-di-C-methyl-, 3-O-methyl-D-glucose, DI-N-Octyl Phthalate (16.444%), 7-[3-Chloro-2-hydroxypropyl] guanine, 1-Naphthalenepropanol,.alp, 1,3,3-trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene, β-Amyrone, and (+)-Helminthogermacrene.

The presence of diverse compounds in the methanolic extract of *R. tetraphylla* suggested its potential therapeutic applications. Compounds such as 1D-1-O-Methyl-myo-inositol, known for addressing metabolic disorders like insulin resistance and diabetes-related complications, and 3-O-methyl-D-glucose, with potential applications in tumour detection and stroke management, indicate the pharmacological significance of *R. tetraphylla*. Additionally, compounds like Butyl cyclohexyl phthalate, 7-[3-Chloro-2-hydroxypropyl] guanine, β-Amyrone, and (+)-

Helminthogermacrene exhibit antioxidant, anticancer, anti-inflammatory, and antiviral activities, suggesting the potential of *R. tetraphylla* in treating various ailments.

The identified compounds contributed to the antioxidant, antimicrobial, anti-inflammatory, and wound healing activities attributed to *R. tetraphylla*. Further research focusing on isolating and characterizing these compounds could lead to the development of novel therapeutic agents with enhanced efficacy and safety profiles. Additionally, exploring the synergistic effects of these compounds and understanding their mechanisms of action can provide valuable insights into the pharmacological properties of *R. tetraphylla* and its potential applications in modern medicine.

The LCMS analysis of the methanol extract of *R. tetraphylla* leaves identified nine significant peaks corresponding to secondary metabolites. These compounds include Oxodecenoic acid, Naringenin, dihydrokaempferide, serpentine, yohimbine, isoreserpinine, darcyribeirine, reserpiline, carapanaubine, and reserpine, each with distinct pharmacological properties and potential therapeutic applications.

The presence of diverse secondary metabolites in the methanolic extract of *R. tetraphylla* leaves underscores its medicinal significance and potential for therapeutic development. Compounds like dihydrokaempferide, serpentine, yohimbine, and reserpine exhibit a range of pharmacological activities including antioxidant, anti-inflammatory, antipsychotic, and antihypertensive properties, indicating the potential of *R. tetraphylla* in treating various ailments such as acute pancreatitis, liver inflammation, erectile disorder, and hypertension.

Methanolic extract of *R. tetraphylla* leaf promising biological activities were subjected to further chromatographic analysis. Column chromatography of the extract produced 59 fractions, which were concentrated and monitored by TLC. Fractions with similar constituents were combined into 10 fractions (F1-F10). TLC analysis of methanol fractions in a HEX:EtOH (4:1) solvent system revealed intense fluorescence. Fraction (F1) was characterized with the help of UV spectrum, FTIR, ^{1}H NMR spectrum, ^{13}C NMR Spectrum and GC Mass spectrum and was identified as butyl cyclohexyl ester.

The identification of these secondary metabolites highlighted the importance of phytochemical research in exploring natural sources for novel drug discovery and development. Further investigations into the mechanisms of action and therapeutic efficacy of these compounds

can lead to the development of new pharmaceutical agents for the treatment of human diseases. Additionally, the wound healing properties attributed to these secondary metabolites evidenced the traditional medicinal uses of *R. tetraphylla* and its potential applications in modern healthcare practices.

The antimicrobial activity of *R. tetraphylla* ethyl acetate and methanolic extracts against Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*) bacteria was evaluated using the agar well diffusion method. Both extracts exhibited significant antimicrobial activity. The methanol extract showed the highest inhibition (19 mm) against *Escherichia coli*. The MIC against *E. coli* was determined using a two-fold broth dilution method, with concentrations ranging from 0.78 µg/mL to 200 µg/mL. The ethyl acetate and methanolic extracts of *R. tetraphylla* leaves and fruit demonstrated varying MIC values against *Escherichia coli*. It confirmed the antimicrobial potential of *R. tetraphylla* extracts, supporting its traditional medicinal uses in treating microbial infections.

The *in-vitro* antioxidant activity of ethyl acetate and methanolic extracts from *Rauvolfia tetraphylla* leaf and fruit was evaluated using various assays, including DPPH, FRAP, and phosphomolybdenum methods.

DPPH scavenging activity of *R. tetraphylla* showed maximum antioxidant activity in methanolic extract of fruit (IC_{50} = 60.37 µg/mL) followed by methanolic extract of leaf (IC_{50} = 62.61µg/mL). In the FRAP assay, the extracts demonstrated the ability to reduce Fe^{3+} to Fe^{2+} ions, with a clear dose-response relationship observed across different concentrations. The methanolic extract from the leaves exhibited the highest antioxidant activity among all the extracts tested. The sequence of antioxidant potential observed was RLM < RL EA < RFM < RF EA. Furthermore, the total antioxidant assay using the phosphomolybdenum method revealed that the methanol extract of the leaf showed maximum (IC_{50} = 63.05 µg/mL) antioxidant activity, followed closely by the methanol extract of the fruit (IC_{50} = 65.37 µg/mL).

This emphasizes the significant antioxidant potential of *R. tetraphylla* leaf and fruit extracts, as demonstrated by their ability to scavenge free radicals and reduce oxidative stress. The presence of alkaloids and flavonoids contributes to the extracted antioxidant properties, making them promising candidates for therapeutic interventions targeting oxidative damage-related

conditions. The increasing reducing power of the extracts in a concentration-dependent manner highlights their potential for wound healing activity.

The anti-inflammatory activity of *R. tetraphylla* leaf and fruit extracts was evaluated using the denaturation of egg albumin method, an *in vitro* technique assessing anti-inflammatory properties by inhibiting albumin protein denaturation. The methanol extracts from both the leaf (IC_{50} = 72.44 µg/mL) and fruit (IC_{50} = 78.04 µg/mL) exhibited the maximum inhibition rate at a concentration of 100 µg/mL, with inhibition increasing gradually with concentrations. This indicated that *R. tetraphylla* leaf and fruit extracts possess anti-inflammatory properties as evidenced by their ability to inhibit protein denaturation. It is warranted to elucidate the underlying mechanisms of action and evaluate the safety and efficacy of these extracts for wound healing applications.

The cytotoxicity and cell viability of ethyl acetate and methanol extracts from *R. tetraphylla* leaves and fruits against Vero cells and 3T3 mouse embryonic fibroblast cells was recorded. The MTT assay was employed to quantitatively assess cell viability, revealing concentration-dependent cytotoxic effects of *R. tetraphylla* extracts. The CC_{50} values, representing the concentration reducing cell viability by 50%, were determined for Vero cells and 3T3 cells. Results indicated that all extracts exhibited CC_{50} values greater than 100 µg/mL, suggesting minimal cytotoxicity and better proliferation activity towards both cell lines within the tested concentration range. This suggested that *R. tetraphylla* extracts, particularly ethyl acetate and methanol extracts from leaves and fruits possess relatively low cytotoxicity and are safe for Vero and 3T3 cell lines. These results support their potential applications in therapeutic wound healing properties without inducing significant cell damage.

Scratch assay was evaluated using Vero and 3T3 cell lines treated with ethyl acetate and methanol crude extracts of *R. tetraphylla*. Wounds were created in cultured cell monolayers treated with test samples and observed after a 24-hour incubation period. Results showed a decrease in wound distance indicating wound closure and cell migration, with more pronounced effects observed in the sample group compared to the control. Wound closure percentages ranged from 63.54% to 72.67% in the sample group with the highest observed with methanol extract of leaves. This determined the wound-healing properties of *R. tetraphylla* extracts, as evidenced by increased cell migration, proliferation and wound closure rates compared to the control group. Both ethyl

acetate and methanol extracts from leaves and fruits exhibited significant effects on wound closure. These results established their potential therapeutic applications in promoting tissue repair and wound healing.

The CAM assay was analysed using the *in-ovo* method to assess the angiogenic potential of ethyl acetate and methanol extracts derived from the leaf and fruit of *R. tetraphylla*. Results indicated that ethyl acetate extracts exhibited limited angiogenic effects while methanolic extracts demonstrated significant angiogenic property. Both leaves and fruits showed substantial impact on angiogenesis suggesting their potential utility in wound healing applications. Qualitative assessment of vascular morphology revealed blood vessels present, and complete enclosure of the collagen/elastin membrane by the chorioallantoic membrane. These extracts exhibited notable effects on angiogenesis, a critical process in wound healing. All extracts displayed significant angiogenesis, suggesting their potential therapeutic utility in wound healing and related applications. Further research is necessary to elucidate the underlying mechanisms and optimize the use of these extracts in clinical settings.

Ethyl acetate and methanolic extracts from leaf and fruit of *R. tetraphylla* were assessed for their ability to enhance resistance against heat stress in *Ceanorhabditis elegans*. Worms were placed on agar plates containing a concentration of 1mg/mL of adaptogen extract for several days, and the number of surviving worms was monitored daily. Results demonstrated that the presence of adaptogens led to higher survival rates among the worms, indicating a protective effect against heat stress. Among the extracts tested, methanol from the leaf exhibited the highest survival rate (22 days), followed by fruit (21 days) extracts. It suggested the adaptogenic properties of *Rauvolfia tetraphylla* extracts and their potential application in mitigating the effects of heat stress-related conditions.

The wound healing potential of ethyl acetate and methanol extracts derived from the leaves and fruit of *R tetraphylla* was evaluated using a Zebra fish model. Fish were divided into control and treated groups, with the treated groups receiving various concentrations of the extracts. Incisional wounds were created on the caudal fins of the fish, and wound healing progression was monitored over a period of 21 days. Results showed that fish treated with 100 µg/mL of ethyl acetate and methanol extracts exhibited maximum fin regeneration at 21st day compared to the control group. Histological analysis through haematoxylin and eosin staining confirmed enhanced

wound healing in the treated groups, with increased neutrophil counts observed compared to the control group. It also revealed various cellular changes associated with wound healing including re-epithelialization, fibroblast activity, and tissue remodelling.

R. tetraphylla extracts are known to contain secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, saponins, polyphenols, and glycosides, which are recognized for their wound healing properties. These bioactive components suggested the potential of *R. tetraphylla* as a therapeutic agent for promoting wound healing. It supports the therapeutic utility of *R. tetraphylla* extracts as natural agents for wound healing applications, warranting further investigation into their mechanisms of action and clinical efficacy.

Tryptophan decarboxylase gene from *Rauvolfia tetraphylla* (*RtTDC*) was investigated for its potential role in the biosynthesis of monoterpenoid indole alkaloids (MIAs) and its implications in wound healing. The *RtTDC* gene sequence was obtained from *R. tetraphylla* and comparative genomic analysis revealed a high degree of sequence similarity (95.39%) with the TDC gene from *R. verticillata*, suggesting conservation within the *Rauvolfia* genus. Structural modelling of *RtTDC* was analysed using comparative protein modelling, utilizing the crystallographically solved structure of *Catharanthus roseus* TDC as a template. The homology model exhibited high-quality features, indicating structural similarities with known TDC proteins. Moreover, phylogenetic analysis highlighted the evolutionary relationships between TDC sequences from different plant species.

Physicochemical properties of *RtTDC* were predicted, revealed its biophysical characteristics, stability, and solubility. The amino acid composition analysis showed the relative abundance of amino acids in the protein sequence, with leucine being the most abundant. Secondary structure analysis indicated a prevalence of alpha helices, further characterizing the conformational features of *RtTDC*. Molecular docking studies with L-tryptophan as the substrate suggested potential interactions and binding affinity between *RtTDC* and its substrate.

Enzymatic decarboxylation activity assays confirmed the role of *RtTDC* in catalysing the conversion of L-tryptophan to tryptamine, a key step in alkaloid biosynthesis pathways. The identification and characterization of *RtTDC* provided valuable insights into the molecular mechanisms underlying alkaloid biosynthesis in *R. tetraphylla*. The investigation of the *RtTDC*

gene from *R. tetraphylla* sheds light on its potential involvement in the biosynthesis of indole alkaloids and its significance in wound healing processes.

Ten biochemical compounds identified through GCMS analysis were subjected to comprehensive ADME (Absorption, Distribution, Metabolism, and Excretion) analysis. These compounds exhibited diverse physicochemical properties, including molecular weight, lipophilicity, water solubility, and pharmacokinetic parameters. All the compounds showed favorable drug-likeness properties with no significant violations of established rules, indicating their potential as pharmaceutical agents. The ADME analysis provides valuable insights into the pharmacokinetic profiles and drug-likeness properties of the GCMS-identified compounds.

Ten biochemical compounds identified through LCMS analysis exhibited diverse physicochemical properties and pharmacokinetic profiles. These compounds showed variations in molecular weight, sp3-hybridized carbon atoms, solubility, GI absorption, BBB permeability, and inhibition potential against CYP enzymes. While most compounds demonstrate favourable drug-like properties with high GI absorption and potential BBB permeability, some exhibited inhibitory effects on various CYP isoforms, which could impact their metabolism and drug interactions. Additionally, compound 10 presents challenges regarding its drug-likeness and alerts for potential liabilities. Despite this, it retains moderate synthetic accessibility, indicating potential for further exploration in drug development. Further optimization and investigation are required to maximize the therapeutic potential of these compounds, particularly in drug development endeavors. Continued research into their metabolic pathways, drug interactions, and *in vivo* efficacy will contribute to their advancement in pharmaceutical research and development.

Molecular docking studies suggested the potential pharmacological implications of *Rauvolfia tetraphylla* in drug design and development. It contributes to our understanding of the molecular mechanisms underlying alkaloid metabolism in *R. tetraphylla* and may have implications for the development of novel therapeutic agents for wound healing applications. However, further research is needed to explore the clinical potential and safety profile of *R. tetraphylla* extracts for practical applications in medicine and biotechnology.

In silico docking studies were conducted using three different protein structures (PDB IDs: 6Y8M, 6B8Y, 1GEN) with various ligands selected for their potential in wound healing

applications. The binding affinities of the ligands were evaluated, and their interactions with amino acid residues within the protein structures were analyzed.

For the protein structure with PDB ID 6Y8M, compounds such as 1,9-Nonanediol (-4.59 kcal/mol), 1D-1-O-Methyl-myo-inositol (-4.29 kcal/mol), and (Z)-2-methylbutanal oxime (-4.12 kcal/mol) exhibited moderate binding affinities, while 7-[3-Chloro-2-hydroxypropyl]guanine (-5.82 kcal/mol), Butyl cyclohexyl phthalate (-7.67 kcal/mol), and Isoreserpinine (-8.25 kcal/mol) showed stronger binding affinities. Even ligands with relatively lower binding affinities, such as Reserpiline (-7.78 kcal/mol) and Carapanaubine (-7.77 kcal/mol), still displayed interactions with critical residues. Similarly, in the protein structure with PDB ID 6B8Y, various ligands formed significant interactions with specific amino acid residues, contributing to their binding affinities. Compounds like 7-[3-Chloro-2-hydroxypropyl]guanine (-6.62 kcal/mol), Butyl cyclohexyl phthalate (-7.70) and Carapanaubine (-8.63 kcal/mol) exhibited strong binding affinities, while others like 1-Naphthalenepropanol (-7.71 kcal/mol), Serpentine (-7.2 kcal/mol), Yohimbine (-7.0 kcal/mol), Isoreserpinine (-7.63), and Reserpiline (-7.1 kcal/mol) also showed notable interactions.

In the context of the protein structure with PDB ID 1GEN, different ligands demonstrated diverse binding energies and interactions with specific amino acid residues. Compounds such as 7-[3-Chloro-2-hydroxypropyl]guanine (-7.16 kcal/mol), 1-Naphthalenepropanol (-7.69), Carapanaubine (-8.22 kcal/mol) displayed strong binding affinities, while others like Butyl cyclohexyl phthalate (-6.29 kcal/mol), Serpentine (-6.83 kcal/mol), Yohimbine (-6.23 kcal/mol), and Isoreserpinine (-5.98 kcal/mol) also formed significant interactions. The docking studies provide insights into the potential ligand-protein interactions within the given protein structures, contributing to a better understanding of their pharmacological significance and potential therapeutic applications in wound healing. Further experimental validation would be necessary to confirm these computational findings and to explore the practical implications of these interactions.

Overall, this multidisciplinary study provides a comprehensive overview of *R. tetraphylla*, spanning from phytochemical characterization to molecular mechanisms, and underscores its potential as a valuable source of therapeutic agents for wound healing and other biomedical applications. Further research building upon these findings could lead to the development of novel drugs would be derived from *R. tetraphylla*.